Supplemental information

Materials and Methods

Assessment of bioactive lipids by LC-MS/MS

After animal sacrifice, the plasma and colon tissues were harvested for LC-MS/MS-based lipidomics analysis. To extract lipid metabolites from plasma, ~250 μL plasma was mixed with deuterated internal standards (500 nM of d_4-6-keto PGF_{1a}, d_4-TXB_2, d_4-PGE_2, d_4-LTB_4, d_{11}-14,15-DHET, d_4-9-HODE, d_8-5-HETE, d_{11}-11,12-EET), then loaded onto pre-washed Waters® Oasis solid phase extraction (SPE) cartridges, washed with 95:5 water/methanol with 0.1% acetic acid, the analytes were eluted with methanol and ethyl acetate, dried using a centrifugal vacuum evaporator, then reconstituted in methanol for LC-MS/MS analysis. To extract lipid metabolites from colon tissues, ~100 mg tissues were mixed with an antioxidant solution (0.2 mg/mL butylated hydroxytoluene and 0.2 mg/mL triphenylphosphine in methanol), deuterated internal standards, and 400 μL extract solution (0.1% acetic acid with 0.2 mg/mL butylated hydroxytoluene in a methanol solution), and then homogenized; the resulting homogenates were kept in −80 °C overnight. After centrifugation of the homogenates, the pellets were washed with methanol (containing 0.1% butylated hydroxytoluene and 0.1% acetic acid) and then combined with the supernatant. The lipid metabolites in the combined solutions were extracted using SPE columns, similar to the description above for the plasma lipid metabolite extraction. The LC-MS/MS analysis was carried out on an Agilent 1200SL HPLC system (Agilent) coupled to a 4000 QTRAP MS/MS (AB Sciex) as described in our previous report. The peaks were identified according to the retention time and specific multiple reaction monitoring (MRM) transitions of the standards of lipid metabolite. The concentrations of the lipid metabolites were calculated against the calibration curve with standards.

Tissue staining

For H&E staining, formalin-fixed tissue was embedded in paraffin (Thermo Fisher Scientific), sliced to 5-μm sections, and dewaxed in serial xylene (Thermo Fisher Scientific), rehydrated through graded ethanol solutions (Pharmco-Aaper), stained with hematoxylin and eosin (Sigma-Aldrich), and examined with a light microscopy. For immunohistochemistry analysis, antigen retrieval was performed by heating the sections in 0.01 M citrate buffer (pH 6.0) to 95°C for 10 minutes. Samples were incubated with anti-PCNA antibody (Dako, catalog no. M087901-2), anti-non-phospho (active) β-Catenin (Ser33/37/Thr41) antibody (Cell Signaling, catalog no. 8814) or anti-Cleaved Caspase 3 antibody (Cell Signaling, catalog no. 9664) overnight at 4 °C. Horseradish peroxidase (HRP)-conjugated secondary antibodies were then applied to the sections, followed by the chromogen 4-diaminobenzidine staining according to the instruction of HRP/DAB (ABC) Detection IHC kit (Abcam). Sections were then counterstained with hematoxylin for 1 minute. Positive expression of PCNA, β-catenin and Caspase 3 were observed under light microscope and were quantified by ImageJ software.

Flow cytometry analysis of immune cell infiltration into colon tissues and MC38 tumors

Distal colon tissues or MC38 tumors were dissected, washed with cold PBS, and digested with Hank's-balanced salt solution (HBSS, Lonza) supplemented with 1 mM dithiothreitol (DTT) and 5 mM EDTA for 2 h at 4 °C. The released cells from mucosa were filtered through 70 μm cell strainer (BD Biosciences) to get single cell suspensions, which were stained with FITC-conjugated anti-mouse CD45 antibody (BioLegend, catalog no. 103107), PerCP/Cy5.5-conjugated anti-mouse F4/80 antibody (BioLegend, catalog no.123127), and isotype control antibody (BioLegend). Zombie Violet™ dye from the Zombie Violet™ Fixable Viability Kit (BioLegend) was used to stain dead cells, according to the manufacturer's instructions. The stained cells were analyzed using BD LSRFortessa™ cell analyzer (BD Biosciences) and data were analyzed using FlowJo software (FlowJo LLC). Gating and cell identification strategies are as follows: briefly, cell doublets and clumps were eliminated using FSC-H vs. FSC-A gating and, debris was eliminated using FSC-A vs. SSC-A. Dead cells were gated out using Zombie Violet™ dye (BioLegend). In our analyses, leukocytes were identified as $CD45^+$ cells and macrophages were identified as $CD45^+$ $F4/80^+$ cells.

qRT-PCR analysis of gene expression in colon tissues, MC38 tumors and cells

For tissues analysis, colon tissues in mice from the same locations or MC38 tumors were ground after freezing in liquid nitrogen and then TRIzol reagent (Ambion) added. For cell assay, the Raw 264.7 or HCT116 cells were treated with 1-100nM 12,13-EpOME or DMSO vehicle in complete medium for 24h, then the medium was decanted, the cells were washed with cold PBS and added with TRIzol reagent. Then, total RNA was isolated from the colon tissues or cells based on manufacturer's instructions of TRIzol reagent. The quality and quantity of the extracted RNA was measured using a NanoDrop Spectrophotometer (Thermo Scientific) and was reverse transcripted into cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to manufacturer's instructions. qRT-PCR was performed in a DNA Engine Opticon system (Bio-Rad Laboratories) with Maxima SYBR-green Master Mix (Thermo Fisher Scientific). The sequences of mouse- and human- specific primers (Thermo Fisher Scientific) were listed in **Table S2**. The results of target genes were normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH or Gapdh)* and compared to these of the control mice or cells using the $2^{-\Delta\Delta Ct}$ method.

Protein extraction and immunoblotting

RAW 264.7 or HCT116 cells were treated with 100 nM 12,13-EpOME or DMSO vehicle in complete medium for 5-15min, then the medium was decanted, the cells were washed with cold PBS and lysed, the cell lysates were resolved using SDS/PAGE and transferred onto a nitrocellulose membrane. The membranes were blocked in Odyssey Blocking Buffer (LI-COR) for 1 h at room temperature and probed with antibodies against phosphor-JNK and JNK (Cell Signaling Technology) and β-actin (Sigma-Aldrich). The membranes were then probed with LI-COR IRDyeR 800CW Goat anti-Rabbit and IRDyeR 680RD Goat anti-Mouse secondary antibodies, and then detected using Odyssey imaging system (LI-COR). Western blot quantification was performed using Image StudioTM Lite Software (LI-COR).

ELISA analysis of cytokines in cell culture medium

The RAW 264.7 cells were treated with 100 nM 12,13-EpOME or DMSO vehicle in complete medium for 72 hours. Then the cell culture media were harvested and the concentrations of cytokines in supernatant were determined using a CBA Mouse Inflammation Kit (BD Biosciences) according to the manufacturer's instruction.